

# Using the AppliFlex ST to Investigate the Effect of CO<sub>2</sub> Concentration on the Expansion of T-cells

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## Introduction

In recent years, adoptive T-cell therapy (ACT) has emerged as a promising way to treat systemic cancers such as Acute Lymphoblastic Leukemia. However, robustness and reproducibility of the manufacturing process remain challenging and it is therefore pivotal to understand the effect that cell culture conditions have on the expansion and differentiation of T-cells. This work investigates the effect of CO<sub>2</sub> on the expansion of T-cells using the bioreactor AppliFlex ST due to the advantages of this single-use system such as lower initial investments, faster setup and reduced cross-contamination risks. The influence of CO<sub>2</sub> is of particular interest with regards to potential allogeneic T-cell therapies and the associated cultivation at the large scale, where CO<sub>2</sub>-removal may become insufficient. Several studies have demonstrated for other mammalian cell types that elevated concentrations of dissolved CO<sub>2</sub> can severely affect the bioprocess (e.g. Brunner et al., 2018, Nguyen Dang et al., 2019), but so far no such studies exist for the expansion of T-cells.

## Materials & Methods

- All experiments were performed in Applikon ST single-use bioreactors with a nominal volume of 500 mL (Figure 1) and a marine impeller.
- For measurements and control of the parameters the my-control was used. The culture conditions are described in Table 1.
- All conditions were replicated with n ≥ 2 donors.
- Bioreactors were supplied with gas mixes of 5%, 10%, and 20% CO<sub>2</sub> balanced with N<sub>2</sub> or O<sub>2</sub>.
- Viable cell concentration and viability were determined using a NucleoCounter NC 3000 (ChemoMetec A/S, Denmark) using Via 1 Cassettes (ChemoMetec A/S, Denmark).
- Concentrations of nutrients and metabolites were measured using a Bioprofile Flex (Nova Biomedical, USA).

Cell Culture Conditions	
Working volume	150 - 300 mL
Gas flow rate	1.0 vvm Overlay
Control of pH	7.2 with CO <sub>2</sub> ↓, 250 mM bicarbonate buffer ↑
Control of DO	25% with Air ↑, N <sub>2</sub> ↓
Mode of operation	Fed-Batch (+100% of initial working volume)
Temperature	37°C
Seeding density	0.4E6 cells mL <sup>-1</sup>
Agitation	200 rpm
Medium	X-Vivo 15 + 5% FBS + 1% Pluronic F68

Table 1 | Culture Conditions for T-cells in the AppliFlex ST



Figure 1 | AppliFlex ST (Applikon Biotechnology)

## Results & Discussion

- Cell growth is near-identical between donors at lower concentrations of CO<sub>2</sub>. Only cells of Donor 14 appeared to be affected by an increased concentration of CO<sub>2</sub>. In this case, growth stalled after 3 days of cultivation and the viability slowly decreased. (Figure 2 and Table 2).
- Interestingly, cells of Donor 14 grown with 20% CO<sub>2</sub> showed an increased specific generation of lactate and consumption of glucose compared to those cells grown at the lower concentrations of CO<sub>2</sub>. An involvement of the pCO<sub>2</sub> on the lactate metabolism has previously been demonstrated for other mammalian cells (Brunner et al., 2018).
- Using the outlined fed-batch process, the glucose concentration was maintained above depletion level and the concentration of lactate and ammonia were maintained below 6 g/L and 3 mmol/L, respectively. Some conditions were potentially glutamine limited in later stages of the cultivation. Further optimisation of the process could include enhanced supplementation with glutamine.

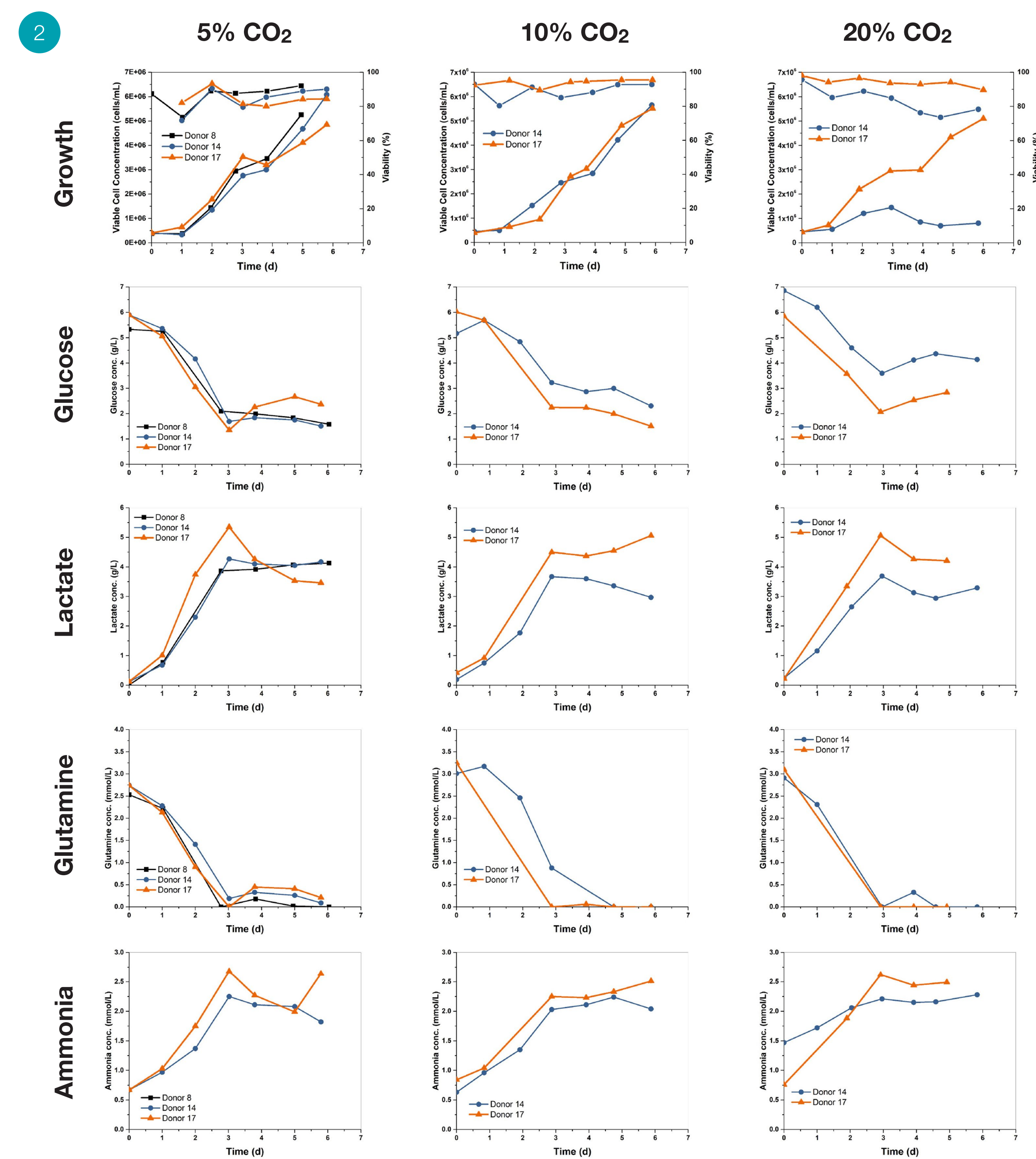
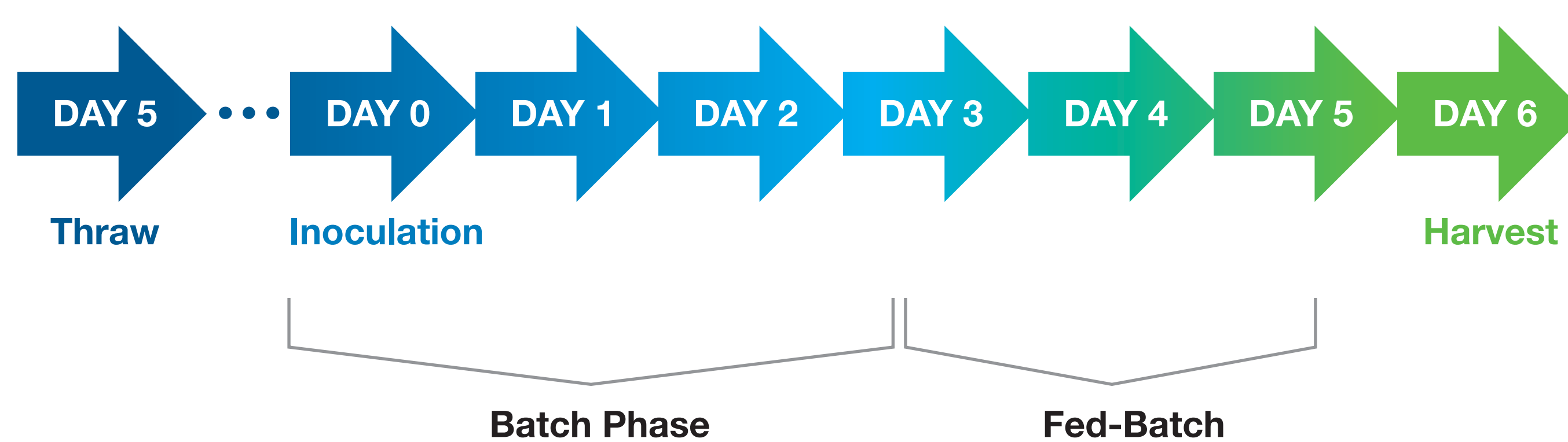


Figure 2 | Growth and metabolism of primary T-cells expanded using the AppliFlex ST single-use vessel. All inflowing gases contained either 5%, 10%, or 20% CO<sub>2</sub>.

## Process Flow



	Fold Expansion		
	Donor 8	Donor 14	Donor 17
5% CO <sub>2</sub>	27,8	30,4	24,3
10% CO <sub>2</sub>	x	24,8	27,9
20% CO <sub>2</sub>	x	3,6	23,2

Table 2 | Final fold expansion of T-cells in the AppliFlex ST under different CO<sub>2</sub> concentrations

## Conclusions

- The AppliFlex ST 500 mL single-use vessels were successfully employed for the expansion of primary T-cells.
- Different concentrations of CO<sub>2</sub> in the inflowing gas were tested for their effect on growth and metabolism of primary T-cells. Concentrations of up to 10% CO<sub>2</sub> were tolerated by all tested donor cells. At 20% CO<sub>2</sub> one of the tested donor cells stalled in growth and showed a change in metabolism, whereas cells of the other donor were unaffected.
- The results highlight that sensitivity to CO<sub>2</sub> toxicity is donor-dependent. With regards to large-scale manufacturing of allogeneic T-cell therapies, it is therefore critical that CO<sub>2</sub> resistance is considered during the cell line selection.

## References

- Brunner M, Doppler P, Klein T, Herwig C, and Fricke J. Elevated pCO<sub>2</sub> affects the lactate metabolic shift in CHO cell culture processes. Eng. Life Sci. 18(3), 204-214 (2018).
- Nguyen Dang A, Mun M, Rose CM, et al. Interaction of cell culture process parameters for modulating mAb atucosylation. Biotechnol. Bioeng. bit.26908 (2019)

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